

recording actually provides a means to "separate" the various permutations of each combination of heteromers. According to these data, combinatorial assembly can provide pores with characteristic responses over a wide range of
5 analyte concentrations.

Other embodiments are within the following claims.

What is claimed is:

1 1. A mutant staphylococcal alpha hemolysin
2 polypeptide comprising a heterologous amino acid, wherein
3 said heterologous amino acid binds an analyte and wherein
4 said polypeptide assembles into a heteroheptameric pore
5 assembly in the presence of a plurality of wild type
6 staphylococcal alpha hemolysin polypeptides.

1 2. The polypeptide of claim 1, wherein said
2 heterologous amino acid occupies a position in a
3 transmembrane channel of said heptameric pore assembly.

1 3. The polypeptide of claim 2, wherein said
2 heterologous amino acid projects into the lumen of said
3 transmembrane channel.

1 4. The polypeptide of claim 2, wherein said
2 heterologous amino acid occupies a position in a stem domain
3 of said polypeptide.

1 5. A staphylococcal alpha hemolysin (α HL)
2 polypeptide comprising at least two non-consecutive
3 heterologous amino acids in a stem domain of said
4 polypeptide, wherein each of said heterologous amino acids
5 binds a metal.

1 6. The polypeptide of claim 5, wherein said amino
2 acids occupy two or more of the following positions of SEQ
3 ID NO:1: 111, 113, 115, 117, 119, 121, 123, 125, 127, 129³,
4 131, 133, 135, 137, 139, 141, 143, 145, 147 or 149.

1 7. The polypeptide of claim 5, wherein said amino
2 acids occupy two or more of the following positions of SEQ
3 ID NO:1: 110, 112, 114, 116, 118, 120, 122, 124, 126, 128,
4 130, 132, 134, 136, 138, 140, 142, 144, 146, 148.

1 8. The polypeptide of claim 5, wherein said
2 polypeptide comprises at least three non-consecutive
3 heterologous amino acids in the stem domain of said
4 polypeptide.

1 9. The polypeptide of claim 5, wherein said
2 polypeptide comprises at least 4 non-consecutive
3 heterologous amino acids in the stem domain of said
4 polypeptide.

1 10. The polypeptide of claim 9, wherein said amino
2 acids occupy positions 123, 125, 133, and 135 of
3 SEQ ID NO:1.

1 11. The polypeptide of claim 10, wherein said
2 polypeptide is 4H.

1 12. The polypeptide of claim 1, wherein said amino
2 acid is selected from the group consisting of Ser Thr, Met,
3 Trp, and Tyr.

1 13. The polypeptide of claim 12, wherein said amino
2 acid is selected from the group consisting of Glu, Asp, Cys,
3 His.

1 14. The polypeptide of claim 13, wherein said amino
2 acid is His.

1 15. A staphylococcal alpha hemolysin (α HL)
2 polypeptide comprising at least two non-consecutive
3 heterologous amino acids in a stem domain of said
4 polypeptide, wherein each of said heterologous amino acids
5 binds an organic molecule.

1 16. The polypeptide of claim 15, wherein said
2 organic molecule is an explosive.

1 17. The polypeptide of claim 15, wherein said amino
2 acids occupy two or more of the following positions of SEQ
3 ID NO:1: 111, 113, 115, 117, 119, 121, 123, 125, 127, 129,
4 131, 133, 135, 137, 139, 141, 143, 145, 147 or 149.

1 18. The polypeptide of claim 16, wherein said
2 polypeptide is 123W/125W.

1 19. The polypeptide of claim 1, wherein said
2 polypeptide further comprises a second heterologous amino
3 acid at a site distant from said stem domain.

1 20. The polypeptide of claim 19, wherein said
2 second heterologous amino acid is a Cys residue at position
3 292 of SEQ ID NO:1.

1 21. A heptomeric pore assembly comprising a mutated
2 staphylococcal α HL polypeptide (MUT), wherein said MUT is an
3 analyte-binding α HL polypeptide.

1 22. The pore assembly of claim 21, wherein said
2 pore assembly is a heptamer having the formula
3 $WT_{7-n}MUT_n$, wherein n is greater than zero and less than
4 seven.

1 23. The pore assembly of claim 21, wherein said
2 analyte-binding α HL polypeptide comprises a heterologous
3 amino acid at a position in a transmembrane channel of said
4 pore assembly, wherein said heterologous amino acid binds a
5 metal.

1 24. The pore assembly of claim 21, wherein said
2 pore assembly is a heptamer having the formula $WT_{7-n}M_n$,
3 wherein n is greater than zero and less than seven.

1 25. The pore assembly of claim 17, wherein said
2 analyte-binding α HL polypeptide is 4H.

1 26. The pore assembly of claim 21, wherein said
2 analyte-binding α HL polypeptide is 123W/125W.

1 27. The pore assembly of claim 25, wherein the pore
2 assembly is a heptamer having the formula
3 $WT_{7-n}4H_n$.

1 28. The pore assembly of claim 27, wherein the pore
2 assembly is a heteroheptamer having the formula WT_64H_1 .

1 29. A digital biosensor device comprising the pore
2 assembly of claim 21.

1 30. The device of claim 29, wherein said analyte-
2 binding α HL polypeptide comprises at least two non-
3 consecutive heterologous amino acids in the stem domain,
4 wherein each of said heterologous amino acids binds a metal.

1 31. The device of claim 29, wherein said analyte-
2 binding α HL polypeptide comprises a chelating molecule in
3 the stem domain of said polypeptide.

1 32. The device of claim 29, wherein said device
2 detects binding of a metal ion to said analyte-binding α HL
3 polypeptide.

1 33. The device of claim 32, wherein said device
2 detects a single channel current.

1 34. The device of claim 32, wherein said device
2 detects a current through two or more channels.

1 35. A method of detecting the presence of an
2 analyte in a test sample, comprising
3 (a) contacting said sample with the pore
4 assembly of claim 21; and
5 (b) detecting an electrical current in a
6 digital mode through two or more channels, wherein a
7 modulation in current compared to a current measurement in a
8 control sample lacking said analyte indicates the presence
9 of said analyte in said test sample. ?

1 36. A method of detecting the presence of an
2 analyte in a test sample, comprising
3 (a) contacting said sample with the pore
4 assembly of claim 21;
5 (b) detecting an electrical current in a
6 digital mode through a single channel, wherein a modulation
7 in current compared to a current measurement in a control
8 sample lacking said analyte indicates the presence of said
9 analyte in said test sample.

1 37. The method of claim 36, wherein said analyte is
2 a metal ion.

1 38. The method of claim 37, wherein said metal ion
2 is Zn(II).

1 39. The method of claim 37, wherein said metal ion
2 is Co(II), Cu(II), Ni(II), or Cd(II).

1 40. A method of identifying an unknown analyte in a
2 mixture of analytes comprising,
3 (a) contacting said mixture with the pore
4 assembly of claim 21;
5 (b) detecting an electrical current in a
6 digital mode through two or more channels to determine a
7 mixture current signature;
8 (c) comparing said mixture current signature to
9 a standard current signature of a known analyte, wherein a
10 concurrence of said mixture current signature and said
11 standard current signature indicates the identity of said
12 unknown analyte in said mixture.

1 41. The method of claim 40, wherein each of said
2 known and unknown analytes is a metal ion.

1 42. A method of identifying an analyte in a mixture
2 of analytes comprising,

3 (a) contacting said mixture with the pore
4 assembly of claim 21;

5 (b) detecting a single channel current in a
6 digital mode to determine a mixture current signature;

7 (c) comparing said mixture current signature to
8 a standard current signature of a known analyte, wherein a
9 concurrence of said mixture current signature and said
10 standard current signature indicates the identity of said
11 unknown analyte in said mixture.

1 43. The method of claim 42, wherein each of said
2 unknown and known analytes is a metal ion.

1 44. The method of claim 43, wherein said metal ion
2 is Zn(II).

1 45. The method of claim 43, wherein said metal ion
2 is Co(II), Cu(II), Ni(II), or Cd(II).